Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 675–678

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Influence of ionising irradiation on clotrimazole in the solid state

B. Marciniec[a,*], K. Dettlaff[a], M. Naskrent[b]

[a] Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, 60-780 Poznań, Grunwaldzka 6, Poland
[b] Department of Medical Physics, Faculty of Physics, A. Mickiewicz University, 60-614 Poznań, Umultowska 85, Poland

ARTICLE INFO

Article history:
Received 14 July 2008
Received in revised form 28 August 2008
Accepted 29 August 2008
Available online 7 September 2008

Keywords:
Clotrimazole
Ionising radiation
Radiolysis products
Free radicals

ABSTRACT

The effect of ionising irradiation on the antifungal drug clotrimazole has been studied. The compound was subjected to ionisation irradiation in the form of high-energy electron beam (25–800 kGy) from an accelerator. Before and after the irradiation the compound was subjected to the EPR, TLC, HPLC and HPLC–MS analysis.

After irradiation with doses 400–800 kGy the colour of the substance was changed from white to cream. Four products of radiolysis appeared in the HPLC chromatogram at 7.7, 4.2, 6.4 and 14.6 min and the active ingredient content decreased to 96.5%. The irradiation with a dose of 25 kGy resulted in the appearance of trace amounts of the product at 7.7 min and free radicals (2.54×10^{14} spins/g). On the basis of the HPLC–MS data, the main product of radiolysis (t_R = 7.7 min) is 1-(9-phenylfluoren-9-yl)-imidazole. Besides traces of (2-chlorophenyl)-diphenylmethanol, other impurities listed in the European Pharmacopoeia (European Pharmacopea, 5th edition, Council of Europe, Strasbourg, France, 2004.) have not been detected. Clotrimazole has been found to show relatively high resistance to ionising irradiation (greater than fluconazole) and probably will be suitable for radiation sterilisation but with doses lower than 25 kGy.

1. Introduction

Clotrimazole (Antifungal® Canesten®, Lotrimin®, 1-[(2-chlorophenyl)-diphenyl-methyl]-imidazole is a popular antifungal drug belonging to the second generation of azole derivatives. It is administered topically in the forms of cream, ointment (also *pro oculi*), solutions, aerosols and vaginal tablets. Substances used for medical therapy must meet certain standards of microbiological and chemical purity, defined by the pharmacopoeias [1,2] and the method of sterilisation by irradiation becomes increasingly popular because of its high efficiency, possibility of application to thermolabile substances and substances in packages [3]. The possibility of its application has to be individually checked because the ionising radiation may have destructive effect on some substances.

Literature from the last two decades has no reports on the radiochemical stability of clotrimazole in solid state, only the information on the stability of its solutions of different pH is available. The reports are consistent in the fact that the compound is stable in the alkaline environment and undergoes decomposition in the acidic solutions. Its acidic hydrolysis leads to a decomposition to (2-chlorophenyl)-diphenylmethanol and imidazole, which has been shown by HPLC [4,5]. The majority of authors used the HPLC method for determination of the radiostability of this compound [6–8] however other methods have also been applied. Abdel-Moety et al. [4] reported the application of TLC-densitometry and HPLC for analysis of clotrimazole degradation *in substantia* and in pharmaceutical preparations.

Radiochemical stability of therapeutic drugs is most often studied by electron paramagnetic resonance (EPR) [9–11]. This method is also used to check if a given substance had been subjected to sterilisation by irradiation as the most often observed effect of irradiation is the appearance of free radicals. Although such verification is not obligatory or recommended by pharmacopoeias, the results can provide valuable information on the degree of damage caused by irradiation.

As reported in our earlier paper [12], the DSC results for clotrimazole irradiated with a dose of 200 kGy *in substantia* show the decrease of the melting point by ~2 °C, suggesting decomposition. However, no colour changes have been found. The aim of this study was to perform a thorough analysis of the character of changes taking place in this substance upon irradiation to confirm or exclude the presence of the radiolysis products.

2. Experimental

2.1. Materials

Clotrimazole, Sigma, LOT 114K0749, content >99.0%; clotrimazole (European Pharmacopeia Standard), LGC Standards; clotrimazole related compound A, (2-chlorophenyl)-diphenylmethanol

* Corresponding author. Tel.: +48 61 8546647; fax: +48 61 8546652.
E-mail address: bmarcin@ump.edu.pl (B. Marciniec).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.08.032

(United States Pharmacopeia Standard), LGC Standards; clotrimazole imp. E, 2-chlorobenzophenone (European Pharmacopeia Standard), LGC Standards; imidazole, Fluka, content >99.5%.

2.2. Methods

2.2.1. Irradiation with E-beam radiation [12]

2.2.1.1. Electron paramagnetic resonance (EPR) spectroscopy. The EPR experiments were carried out for non-irradiated and irradiated samples, in standard EPR quartz sample tubes from Wilmad. The measurements were performed with a Bruker EPR EMX-10 spectrometer working at 9.4 GHz (X-band) at room temperature (293 K). The sensitivity of the spectrometer is 1×10^{10} spins/g. Induction of the magnetic field was measured to an accuracy of 0.001 mT. Microwave frequency was measured to an accuracy of 0.001 GHz.

2.2.2. Thin layer chromatography (TLC)

Kiesegel 60 F_{254} plates of dimensions 5.0 cm × 20.0 cm and 20.0 cm × 20.0 cm were used. The mobile phases were ethylene chloride:acetone 1:1(phase A) and toluene:methanol 4:1 (Phase B). 50 μl of 2.5% solution clotrimazole (1.25 mg of substance) were spotted on the plate. The spots were detected with a quartz lamp working at λ = 254 nm.

2.2.3. High-performance liquid chromatography (HPLC)

The HPLC system consisted of a Waters Model 616 solvent pump system equipped with a Photodiode Array UV–vis Waters 996 detector set at 220 and 260 nm. Chromatographic separation was performed with a Xterra C_{18} reverse phase column (4.6 mm × 250 mm, 5 μm particle size). The mobile phase consisted of phosphate buffer (20 mM KH_2PO_4 in a 9:1 water–acetonitrile mixture)–acetonitrile (50:50, v/v), at a flow rate of 1.0 ml min^{-1}. The separation was conducted at room temperature. The run time was 20 min. The precision of the HPLC method was characterised by relative standard deviation of 1.84%. The quantification limit was 0.42 mg l^{-1} and the limit of detection was 0.14 mg l^{-1}.

The content of clotrimazole was calculated by reference to the standard. The difference in the content between the irradiated clotrimazole and the calculated content of clotrimazole brought the information on the loss of the active substance as a result of irradiation, and it was the sum of the content of the radiolysis products. The content of the unidentified radiolysis products was determined on the basis of the equivalent detector response [13] assuming the area of the peak in the spectrum of the clotrimazole standard as 100% (detector set at 260 nm).

2.2.4. High-performance liquid chromatography–mass spectrometry (HPLC–MS)

The HPLC–MS analyses were performed using a Waters/Micromass ZQ mass spectrometer, the instrument was coupled to a Waters model 2690 HPLC pump (Milford, MA, USA), detector Photodiode type 996 (Waters), the sample solutions were injected into the Symmetry C18 RP column (150 mm × 4.6 mm, Waters). A mobile phase of acetonitrile–water (75:25, v/v) was applied. The electrospray (ESI) source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 30 V.

3. Results and discussion

According to the organoleptic analysis, the irradiation of clotrimazole with high doses (400–800 kGy) cause a small change in its colour from white to beige.

The EPR spectra showed no signals from the non-irradiated crystalline clotrimazole. Fig. 1 shows the EPR spectrum of irradiated clotrimazole. The intensity of the EPR spectra after irradiation

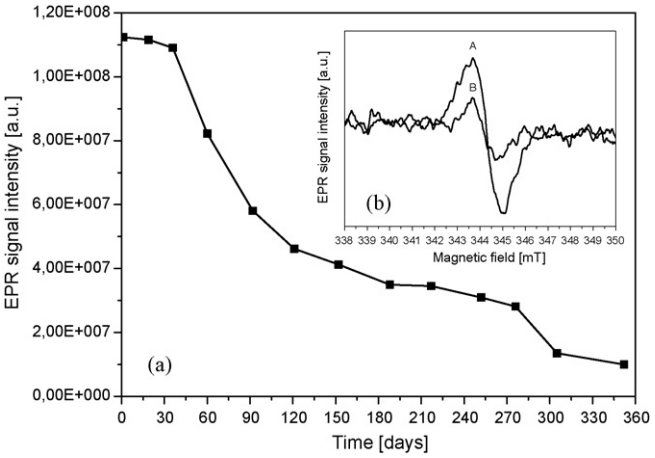

Fig. 1. (a) The decay of EPR signal intensities for clotrimazole irradiated with a dose 25 kGy and stored at room temperature. (b) EPR spectra of clotrimazole after irradiation with a dose 25 kGy: after 1 day (A), after 352 days (B).

is very weak. The EPR spectra were measured after irradiation and subsequently several times over a period of 1 year. Changes over time were observed in the line intensities (Fig. 1). The stability of the radicals in the irradiated clotrimazole samples was also studied. The signal intensity decay data obtained for a sample irradiated at the dose 25 kGy were used to get the decay characteristics of the radicals. The variations in the signal height

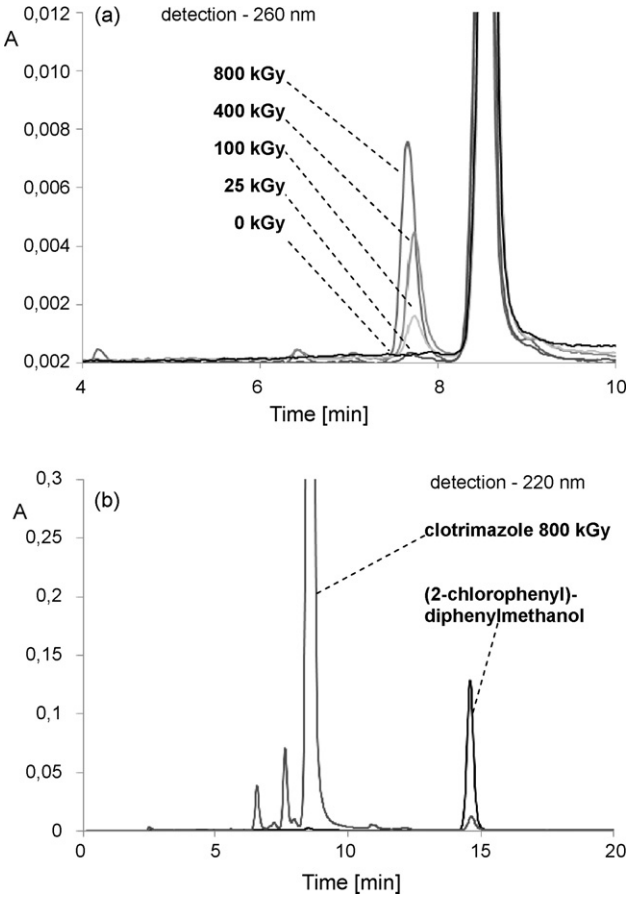

Fig. 2. HPLC chromatograms of: (a) clotrimazole before and after irradiation (detection at 260 nm) and (b) irradiated clotrimazole and (2-chlorophenyl)-diphenylmethanol (detection at 220 nm).

Table 1
The UV and HPLC parameters of radiolysis products of clotrimazole after irradiation with 800 kGy.

Compound	HPLC t_R (min)	λ_{max} (nm)	Content (%)	
Clotrimazole	8.5	261	96.5	
Product I, 1-(9-phenylfluoren-9-yl)-imidazole	7.7	232 and 284	24.0[a]	3.5
Product II	6.4	200	1.9[a]	
Product III	4.2	230	1.2[a]	
Product IV, (2-chlorophenyl)-diphenylmethanol	14.6	200	Not determined[b]	

[a] Equivalent detector response (100% = peak area of clotrimazole reference standard, detector set at 260 nm).
[b] Identification at 220 nm.

of different resonance lines over a period of 352 days are given in Fig. 1. Over 1 year of storage a decrease of the signal intensity for clotrimazole was 81%. In 1 year the concentration of free radicals for irradiated clotrimazole decreased from 2.54 to 0.23×10^{14} spins/g.

The chromatographic analysis by the TLC method performed with the four mobile phases proved that before irradiation clotrimazole was chromatographically pure, while after the irradiation with the doses 25–400 kGy the results indicated the presence of one product of radiolysis of $R_f = 0.83$ (mobile phase A) or $R_f = 0.52$ (mobile phase B). The presence of the second product of radiolysis characterised by $R_f = 0.89$ (mobile phase A) was observed only after the irradiation with 800 kGy.

The analysis by high-performance liquid chromatography (HPLC) confirmed and supplemented the TLC results. The trace amounts of the main product of radiolysis of $t_R = 7.7$ min were detected already after the irradiation with 25 kGy (Fig. 2, Table 1), while in the clotrimazole samples irradiated with 800 kGy the presence of three additional products ($t_R = 4.2$, 6.4 and 14.6 min) of radiolysis was observed, also in trace amounts. Quantitative analysis has not shown a loss in the clotrimazole content after the irradiation with the doses 25–100 kGy; only the irradiation with higher doses brought about a decrease in the content of clotrimazole to 97.7% for 400 kGy and 96.5% for 800 kGy. The UV spectrum of the main product of radiolysis showed two maxima at 232 and 284 nm (Fig. 3), while the maxima assigned to the two other radiolysis products were shifted towards shorter wavelengths and absorbed in the range 200–240 nm.

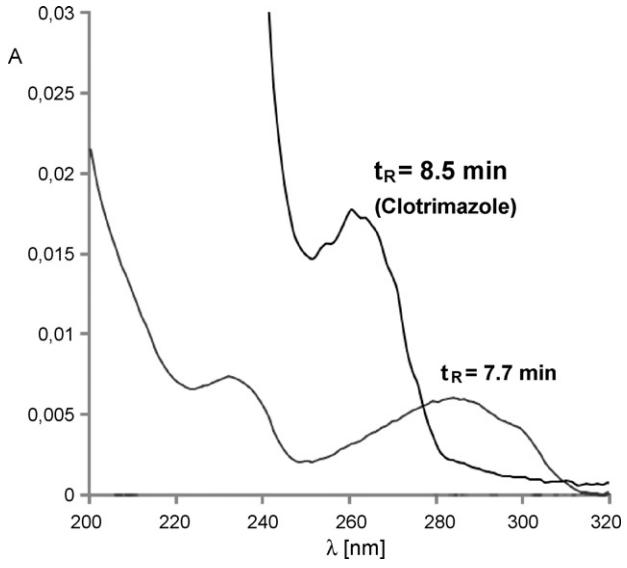

Fig. 3. UV spectrum of radiolysis products of clotrimazole (800 kGy) (solvent: HPLC mobile phase).

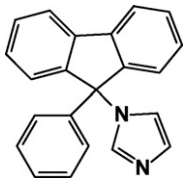

Fig. 4. The structural formula of 1-(9-phenylfluoren-9-yl)-imidazole.

An attempt was made to identify the radiolysis products by comparison of their spectra and retention times with those of the three compounds listed in the pharmacopoeia [1] as potential contaminants of clotrimazole (imidazole-(2-chlorophenyl)-diphenylmethanol and 2-chlorobenzophenone). The retention times of these compounds were 2.4, 14.6 and 9.2 min, respectively. The retention time of only one compound was in agreement with the retention time of one radiolysis product (Fig. 2b.). This product, forming in trace amounts, was (2-chlorophenyl)-diphenylmethanol at 14.6 min, appearing also upon acidic hydrolysis.

On the basis of the HPLC–MS study, the main radiolysis product at 7.7 min, showed the presence of ions at 309 and 310 m/z (ES+). Assuming that these ions are formed by proton attachment, the main radiolysis product of clotrimazole should have the molecular mass of 308 units. The lack of the characteristic isotope ions typical of chlorine excludes the presence of this element in the structure of this product. Moreover, its UV spectrum is similar to that of fluorene, on the basis of the above it was concluded that the main radiolysis product of clotrimazole was 1-(9-phenylfluoren-9-yl)-imidazole ($M = 308.38$, molecular formula: $C_{22}H_{16}N_2$), see Fig. 4.

4. Conclusions

The above presented results have shown that the physicochemical properties of clotrimazole are not changed upon sterilisation by ionising irradiation with doses ranging from 25 to 100 kGy. Although upon irradiation with these doses the presence of free radicals, trace amounts of the radiolysis products are detected, but a decrease in the content is observed by HPLC only after the irradiation with high doses. Upon irradiation with doses $\geq$100 kGy the decomposition of clotrimazole did not exceed 1%, which indicates that the radiochemical stability of the compound is high enough to permit its safe sterilisation by irradiation with doses in the range 25–50 kGy. This substance has been found to be definitely more stable than fluconazole, another antifungal azole derivative [14]. The presence of trace amounts of the radiolysis product 1-(9-phenylfluoren-9-yl)-imidazole already after the irradiation with 25 kGy, indicates the need for a further study to establish the pharmacological and toxic effect of this compound. The analytical methods applied in the study: EPR, TLC and HPLC are useful for determination of changes taking

place upon irradiation as they permit detection of absorbance changes, presence of free radicals and radiolysis products whose presence has been earlier predicted on the basis of the melting point depression observed by DSC method [12]. The results have illustrated the usefulness of the DSC method for monitoring changes taking place in drugs upon sterilisation by irradiation [15,16].

Acknowledgment

This research was supported by the Polish State Committee for Scientific Research (KBN Grant no. 165433).

References

[1] European Pharmacopea, 5th edition, Council of Europe, Strasbourg, France, 2004.
[2] Pharmacopoea Polonica, Editio VI, pp. 60–62, 114–131, 191–192, 2002.
[3] N.G.S. Gopal, Radiat. Phys. Chem. 12 (1978) 35–50.
[4] E.M. Abdel-Moety, F.I. Khattab, K.M. Kelani, A.M. Abou Al-Alamein, Farmaco 57 (2002) 931–938.
[5] J.G. Hoogerheide, B.E. Wyka, in: K. Florey (Ed.), Clotrimazole in Analytical Profiles of Drug Substance, vol. 11, Academic Press, 1982, p. 225 pp.
[6] B.A. Mousa, N.M. El-Kousy, R.I. El-Bagary, N.G. Mohamed, Chem. Pharm. Bull. (Tokyo) 56 (2008) 143–149.
[7] R. Hajkova, H. Sklenarova, L. Matysova, P. Svecova, P. Solich, Talanta 73 (2007) 483–489.
[8] J.G. Hoogerheide, S.H. Strusiak, C.R. Taddei, E.R. Townley, B.E. Wyka, J. Assoc. Off. Anal. Chem. 64 (1981) 864–869.
[9] J.P. Basly, I. Longy, M. Bernard, Int. J. Pharm. 154 (1997) 109–113.
[10] M. Polat, M. Korkmaz, Int. J. Pharm. 255 (2003) 209–215.
[11] C. Aydas, M. Polat, M. Korkmaz, Radiat. Phys. Chem. 77 (2008) 79–86.
[12] B. Marciniec, M. Kozak, K. Dettlaff, J. Therm. Anal. Cal. 77 (2004) 305–317.
[13] International Conference on Harmonization (ICH), Guidance for Industry, Impurities in new drug substances Q3A, June 2008.
[14] B. Marciniec, K. Dettlaff, E. Jaroszkiewicz, J. Bafeltowska, J. Pharm. Biomed. Anal. 43 (2007) 1876–1880.
[15] B. Marciniec, M. Kozak, L. Wachowski, M. Ogrodowczyk, J. Therm. Anal. Cal. 73 (2003) 473–485.
[16] B. Marciniec, M. Kozak, M. Naskrent, K. Dettlaff, M. Ogrodowczyk, M. Stawny, L. Wachowski, J. Therm. Anal. Cal. 88 (2007) 337–342.